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13. ABSTRACT (Maximum 13 lines, use the attachment if necessary)
The overarching goals in this DURINT project have been: 1. Combinatorial selection and post-selection engineering polypeptides that have specific affinity to inorganic surfaces (GEPI); 2. Understanding the nature of protein molecular binding on inorganic surfaces using experimental and theoretical tools; 3. Use these polypeptides as molecular tools to assemble and make nanostructures, and 4. To develop hybrid complex materials with nanoarchitectures, composed of peptides, polymers and nanoinorganics for electronic, photonic, and magnetic applications The accomplishments included: Selection of GEPI using phage and cell surface display protocols; Post-selection engineering for tailored binding and improved functionalities; *In-silico* design of Peptides; GEPI binding characteristics using FM, SPR, QCM, and AFM; Assessing chemical binding & conformation of using XPS, TOF-SIMS and ss-NMR; Development of GEPI-designer protein conjugates and assemblers/immobilizers; Conjugation of GEPI and functional monomers designed and synthesized; Modeling of molecular conformation of GEPI on solids; Permissive site analysis on DNA binding and fluorescent proteins for clones for genetic engineering; Synthesis of inorganics for control of size and composition using GEPIs; Control inorganic architecture and immobilization using GEPIs & DNA templates; Development of protein-based nanomasks, GEPI and viral based templates for nanophotonics, including potential use by the DoD applications.

14. SUBJECT TERMS
Biomimetics, combinatorial biology, inorganic-binding peptides, molecular erectors, nanophotonics, nanoelectronics, genetic engineering, nature of molecular recognition, bioinformatics, protein-based materials, self-assembly

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UW-DURINT Final Report (February 2007)

Genetically Engineered Proteins for Functional Nanoinorganics

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PROGRESS REPORT

1. RESEARCH PROGRESS: Biomimeticists, inspired by biological structures and their functions, focused traditionally on emulating or duplicating biosystems using mostly synthetic components and following traditional approaches. With the recent developments of molecular and nanoscale engineering in physical sciences, and the advances in molecular biology, biomimetics is now entering the molecular scale. In this DURINT research, by combining Nature's molecular tools with synthetic nanoscale constructs, molecular biomimetics is emerging as a hybrid methodology. Our proposed program in this DURINT project has been to:

(1) Develop genetic engineering protocols to select polypeptides with affinity to inorganic surfaces and generate combinatorial peptide-libraries using innovative screening methodologies to optimize genetic mutant selection (this will include evolution of functional protein subunit structural sequences with species-specific binding selectivity);

(2) Characterize protein/inorganic interface structures and properties using both spectroscopic techniques and molecular modeling based on molecular dynamics calculations;

(3) Develop protocols for the formation of nanostructural units (quantum dots, nanowires, and tubes) and demonstrate their controlled assembly into useful 1-, 2-, and 3-D structures, and

(4) Identify possible practical molecular-manufacturing-based approaches to making functional nanostructures for device applications (e.g., multispectral arrays, improved photovoltaics, hybrid molecular switches, and novel sensors).

Our overall goal is to understand, duplicate, and improve upon natural processes through combinatorial genetic engineering techniques to select proteins with high specificity to inorganic surfaces, and then use bioinspired pathways to manufacture a new generation of nanostructured multifunctional materials for DoD's nanotechnology applications.

Recognizing the necessity based on the nature of the proposed research at the cross-sections of biology and materials sciences, we formed a strong multidisciplinary/multi-institutional group with expertise in genetic engineering and biochemistry (Baneyx & Traxler), surface science (Ohuchi), materials sciences (Jen, Schwartz, & Sarikaya), and electro-optical materials and devices (Jen, Baneyx, and Sarikaya)), and a strong collaborative effort with the Microbiology (Ram Samudrala, UW) and Istanbul Technical University (Candan Tamerler). During the progress of the last one year, we developed partnerships with government-based laboratories including Natick Soldier Center (Charlene Mello), Air Force Laboratory (Morley Stone, WPAFB), Pacific Northwest National Labs (Eric Ackerman), and NASA Ames Lab, CA (Jonathan Trent). The proposed projects involve strong student participation at the post-doctoral, graduate, and undergraduate levels, some of who have multiple supervisors working in several PI labs. Several high school and international visiting students are also involved in the research activities (see DURINT web-site: http://depts.washington.edu/bionano).

2. SUMMARY AND THE HIGHLIGHTS OF THE RESEARCH RESULTS FOR THE PERIOD MAY 1, 2001 – NOVEMBER 30, 2006:

1. **Implementation of Combinatorial Mutagenesis**: Both cell-surface display (CSD) and phage display (CD) protocols have been successfully implemented; noble metals (Au and Ag, in addition to Pt and Pd previously selected) and oxides (hydroxyapatite, titania, alumina, silica, in addition to Cu_2O and ZnO), minerals (mica and graphite) have been used for selective binding of polypeptides. Both protocols are brought into routine operation for selection of other potential functional inorganics. New terminology has been developed to call these as genetically engineered peptides for inorganics (GEPI). (Baneyx, Tamerler, Schwartz, Sarikaya, Traxler);
2. **Development of Post-selection Genetic Engineering for Tailoring inorganic-binding peptides:** Using phage and cell surface proteins as the express vector, the group has been able to design inorganic-binding peptides in constraint, linear and multimerized forms. Genetic protocols for alanine scan has also been developed (Tamerler and Sarikaya)
3. **Form-Function Relation at the Molecular Scale: Demonstration of a Self-assembly of a GEPI – Structure and Kinetics**: Self-assembly of a GEPI with a long-range crystallographic organization (supramolecular organization) has been demonstrated on an atomically flat inorganic surface and its structure confirmed using ss-NMR molecular structure (Evans, Tamerler, and Sarikaya).
4. **The first Demonstration of Molecular Erector sets, a Significant Step in Nanotechnology Platforms**: Biotinylated-GEPI (bio-GBP, bio-PtBP, and bio-QBP, for gold-, platinum-, and quartz-binding proteins) has been developed that act as functional molecular substrate by assembling streptavidin (SA) or SA-functionalized functional molecules. Many of similar biofunctional constructs have been developed (Tamerler, Evans, & Sarikaya);
5. **Designer proteins have been developed with added functionality through GEPI**; both enzymes (e.g., maltose binding protein), and DNa-binding proteins (Lac-I) have been developed with genetic fusion of GEPIs. These molecular substrates have been demonstrate to directed immobilize inorganic nanoparticles, an essential first step towards self-assembly of complex (hybrid) structures. (Baneyx, Schwartz, Traxler, and Sarikaya);
6. **Crystalline assembly of S-layer protein as nanomasks for electrochemical fabrication of metallic nanostructures**. S-layer proteins from *C. crescentus* have been assembled with a crystalline pattern on Au(111) under specific conditions of buffer and protein concentration and metals (Au, Ag, Pt, Pd, Cu, Ni, etc.) have been grown electrochemically using the assembled S-layer as nanomasks (Schwartz, Baneyx, and Sarikaya);
7. **Biofabrication of material using genetically selected and designed peptides**, including hydroxyapatite, silica, and cuprous oxide (Tamerler, Schwartz, Baneyx, and Sarikaya);
8. **Design, synthesis, and controlled assembly of hybrid molecules for nanophotonics:** A series of molecules have been designed and synthesized based on their molecular structures (to improve intermolecular interactions), these were further expanded to include side chains (both as functional units as well as intermolecular interactions), and hybridized with GEPIs. Hybrid molecular assembly (aided by micro-contact printing) has been demonstrated by AFM. (Jen & Sarikaya);

3. MAJOR IMPLICATIONS OF THE RESULTS FROM THE THIS PERIOD:

Throughout this research in the period May 1, 2001 through Nov 30, 2006 (extended), the research has produced significant results (highlighted above in eight points), reflecting that we established a viable collaborative polydisciplinary group as well as demonstrating significant research progress that could have wide potential impact. The establishment of expression technologies as well phage- and cell-surface-based in vivo display, is a significant first step in for genetic tailoring of peptides (second generation) that have already been selected (first generation) The UW-DURINT has continued to demonstrate its operation and functioning of the group as a polydisciplinary research entity involving, in an integrated manner, both physical and biological sciences. Self-assembly of genetically engineered polypeptides for inorganics (GEPI) on an inorganic surface and its use as a molecular erector set to further assemble functional proteins in a controllable way are fundamental demonstrations that GEPI can actually now be used as functional molecular erectors, assemblers, and synthesizers set, For the first time, CD and NMR structures of GEPIs (gold-, Pt-, silica, and hydroxyapatite-binding peptides) have been determined, and related to their functions (binding and assembly). Again, for the first time, Bioinformatics tools have been adapted in the molecular evolution of GEPIs and novel bioinformatics-tools have been developed with unprecedented opportunities in molecular building block design as fundamental tools in upcoming revolution in molecular technologies and medicine. One important implication is that these well-ordered, genetically engineered proteins can now be used effectively for molecular technologies, including nanobiotechnology (for example, advanced sensors) and in proteomics.. Furthermore, our chemist colleagues have developed protocols to design, synthesize, and assemble monomolecular functional (electronic) units that were shown (by the materials and structural group) to assemble selected substrates in long-range crystalline patterns. We also showed, for the first time, that not only the molecular architecture of the individual units, but also their 2D ordered assembly affected nanoscale-related functional characteristics (for example, I/V behavior, using STS). One of the major areas of applications of the GEPi has been their utility as linkers and molecular erectors, especially in the immobilization of enzymes, functional proteins, and other biomolecular entities. Finally, hybrid molecular units, including synthetic and biological segments, have been designed and synthesized, a very rapid progress in this field using both microcontact printing and dip-pen nanolithography, blazing new paths towards microarray and nanoarray technologies. The following section lists short synopses of these developments (and their associated publications) during this period.

4. SHORT SYNOPSES OF RESEARCH PROGRESS:

a. The First Concept of Biomimetics: a. Proteins, through their unique and specific interactions with other macromolecules and inorganics, control structures and functions of all biological hard and soft tissues in organisms. Molecular biomimetics is an emerging field in which hybrid technologies are developed by using the tools of molecular biology and nanotechnology. Taking lessons from biology, polypeptides can now be genetically engineered to specifically bind to selected inorganic compounds for applications in nano- and biotechnology. This review discusses combinatorial biological protocols, that is, bacterial cell surface and phage-display technologies, in the selection of short sequences that have affinity to (noble) metals, semiconducting oxides and other technological compounds. These genetically engineered proteins for inorganics (GEPIs) can be used in the assembly of functional nanostructures. Based on the three fundamental principles of molecular recognition, self-assembly and DNA manipulation, we highlight successful uses of GEPI in nanotechnology. **b. Materials Assembly and formation using Engineered Polypeptides** - Molecular biomimetics can be defined as mimicking function, synthesis, or structure of materials and systems at the molecular scale using biological pathways. Here, inorganic-binding polypeptides are used as molecular building blocks to control assembly and

formation of functional inorganic and hybrid materials and systems for nano-and nanobiotechnology applications. These polypeptides are selected via phage or cell surface display technologies and modified by molecular biology to tailor their binding and multifunctionality properties. The potential of this approach in creating new materials systems with useful physical and biological properties is enormous. This mostly stems from molecular recognition and self-assembly characteristics of the polypeptides plus the added advantage of genetic manipulation of their composition and structure. In this review, we highlight the basic premises of molecular biomimetics, describe the approaches in selecting and engineering inorganic-binding polypeptides, and present examples of their utility as molecular linkers in current and future applications.

*See, M. Sarikaya, C. Tamerler, A.K.Y. Jen, K. Shulten and F. Baneyx, "Molecular biomimetics: nanotechnology through biology", Nature Materials, **2** (9), 577-585 (2003); and M. Sarikaya, C. Tamerler, D.T. Schwartz and F. Baneyx, "Materials Assembly and Formation using Engineered Polypeptides", Annu. Rev. Mater. Res., **34**, 373-408 (2004).*

b. Cell Surface Genetic Selection: Identification and Characterization of Cu_2O-and ZnO-Binding Polypeptides by Escherichia coli Cell Surface Display: Toward an Understanding of Metal Oxide Binding - We have used the FliTrx cell surface display system to identify disulfide-constrained dodecapeptides binding to the semiconducting metal oxides Cu_2O and ZnO. Sequence analysis of the inserts revealed that the two populations exhibit similar, yet subtly different patterns of amino acid usage. Both sets of binders were enriched in arginine, tryptophan, and glycine with a higher degree of positional preference in the case of Cu_2O binders. Tyrosine, proline, and serine were underrepresented in both populations. Peptides binding electrodeposited Cu_2O or ZnO with high avidity could be subdivided into two classes based on pI and hydrophilicity. In the hydrophilic and positively charged Class I binders, the Arg–X–X–Arg tetrapeptide appears to be implicated in metal oxide binding, whereas Arg – Arg and Arg – Lys pairs allow for discrimination between Cu_2O and ZnO. Molecular dynamics simulations of the disulfide-constrained peptides suggest that the aforementioned motifs are important to properly orient two basic residues that are likely to contact the metal oxides. The implications of our results in understanding the rules governing the interaction between peptides and inorganic compounds and in their use for the design of hybrid nanoarchitectures are discussed.

*See, C. K. Thai, H. Dai, M. S. R. Sastry, M. Sarikaya, D. T. Schwartz and F. Baneyx, "Identification and Characterization of Cu_2O- and ZnO-Binding Polypeptides by Escherichia coli Cell Surface Display: Toward an Understanding of Metal Oxide Binding", Biotechnology and Bioengineering, **87** (2), 129-137 (2004).*

c. Molecular Biomimetics as a New Paradigm in Implementation of Mother Nature's Molecular Ways in Practical Engineering: In Nature, proteins are the machinery of biological systems that accomplish many functions through their specific recognition and interactions with other proteins and biomolecules in the simplest virus to single-celled and multicellular organisms. Biomolecule-material interaction is accomplished via molecular specificity leading to the formation of controlled structures and functions at all scales of dimensional hierarchy. Through evolution, molecular recognition and, consequently, functions developed through successive cycles of mutation and selection. Using biology as a guide, we can now understand, engineer, and control peptide-material interactions and exploit these to tailor novel materials and systems for practical applications. We adapted combinatorial biology protocols to display peptide libraries either on cell surface or on phage to select short peptides specific to a variety of practical materials systems. Following the selection step, we determine the kinetics and stability of peptide binding experimentally, understand bound peptide structure via modeling, and its assembly via

atomic force microscopy. The peptides are further engineered to have multiple repeats or their amino acid sequences varied to tailor their function. Both nanoparticles and flat inorganic substrates, containing multi-materials patterned at the nano- and micro-scales, are used for self-directed immobilization of molecular constructs. The molecular biomimetic approach opens up new avenues for the design and utilization of multifunctional molecular systems with wide ranging applications from tissue engineering, drug delivery, biosensors, to nanotechnology, and bioremediation. Here we describe lessons from biology with examples of protein-mediated functional materials, peptide selection and engineering with affinity to inorganics, and demonstrate potential utilizations in materials science, engineering, and medicine, and describe future prospects.

See publications in C. Tamerler and M. Sarikaya, "Molecular biomimetics: Utilizing Nature's molecular ways in practical engineering", *Acta Biomaterialia*, **3**, 289-299 (2007); Review, *and C. Tamerler, T. Kacar, D. Sahin, H. Fong and M. Sarikaya, "Genetically engineered polypeptides for inorganics: A utility in biological materials science and engineering",* Materials Science and Engineering C, ***27****, 558-564, (2007)*

d. Adsorption Kinetics of GEPI using Surface Plasmon resonance Spectroscopy and Quartz Crystal Microbalance: The adsorption kinetics of an engineered gold binding peptide on gold surface was studied by using both quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) spectroscopy systems. The gold binding peptide was originally selected as a 14-amino acid sequence by cell surface display, and then engineered to have a 3-repeat form (3R-GBP1) with improved binding characteristics. Both sets of adsorption data of 3R-GBP1 were fit to Langmuir models to extract kinetics and thermodynamics parameters. In SPR, the adsorption onto the surface shows a biexponential behavior and this is explained as the effect of bimodal surface topology of the polycrystalline gold substrate on 3R-GBP1 binding. Depending on the concentration of the peptide, a preferential adsorption on the surface takes place with different energy levels. The kinetic parameters (e.g., $K_{eq} \sim 10^7$ M^{-1}) and the binding energy (~ -8.0 kcal/mol) are comparable to synthetic-based self-assembled monolayers. The results demonstrate the potential utilization of genetically engineered inorganic surface specific peptides as molecular substrates due to their binding specificity, stability, and functionality in aqueous-based environment.

*See, for example, C. Tamerler, M. Duman, E. Venkatasubramanian, E. E. Oren, and M. Sarikaya, "Adsorption kinetics of a gold-binding protein by surface plasmon resonance spectroscopy and quart crystal microbalance," Langmuir, **22** (18) 7712-7718 (2006).*

e. Cross-Selectivity of GEPIs on Inorganic Substrates: Binding kinetics of platinum, silica and gold binding peptides were investigated using surface plasmon resonance spectroscopy (SPR). Platinum and silica binding peptides were selected using phage display technology. Platinum binding septa-peptides were originally selected as loops constrained via cysteines. The dodecapeptide silica binding sequences were genetically selected using a linear phage library. Gold binding peptide was selected using cell surface display using Lamb vector on *E. coli*. Platinum binding peptides were engineered into linear (exclusion of cysteines) and three-repeat cyclic form, while the silica binding peptides in to three repeat linear form. Gold binding peptide (GBP1) is a 14 amino acid long and it is engineered to have three linear repeats. Two different binders, originally characterized to be strong binders to their respective materials, i.e., Pt and silica, were investigated each for platinum (PtBP1 and PtBP2) and silica (QBP1 and QBP2), respectively. The focus of the research was to assess quantitatively the specific affinity of each of the peptides to its material of selection compared to the other two materials as well as the effect of the number of repeats. The results demonstrate knowledge how does engineering affects the

binding affinity in each of the inorganic binding peptide sequence. Depending on the amino acid sequence binding affinity for inorganic surfaces may change by increasing the repeating units. The kinetic numbers also suggest that inorganic binding peptides are good candidate to be utilized instead of other chemically created linker structures like silanes and thiols. The results suggest that binding affinity can be tuned up by engineering of the peptides and inorganic binding peptides are good candidates to be utilized as self assembled layers with their unique surface recognition properties.

See, U. Seker, B. Wilson, D. Sahin, C. Tamerler, and M. Sarikaya, "Cross-specificity of inorganic binding peptides," Langmuir, accepted (2007).

f. Materials Specificity and Directed Assembly of a Gold Binding Peptide: Adsorption studies of a genetically engineered polypeptide, gold binding protein (GBP1), were carried out using quartz crystal microbalance to quantify specificity of adsorption on gold compared to that on noble metal platinum. Cross-specificity experiments were also carried out using biotinylated GBP1 on gold-micropatterned silicon dioxide, in the form of metal strips over the semiconductor wafer. The preferential adsorption of GBP1 on gold regions over the substrate SiO_2 was delineated by using streptavidin-conjugated quantum dots (SA-QD) which showed reversal of contrast on the metal regions. To compare adsorption of biotinylated GBP1 between the two noble metals, gold and platinum, we prepared silicon dioxide substrate that was micropatterened with the two metal squares. Again, SA-conjugated QDs have assembled on the Au regions preferentially compared to the platinum. These experiments not only demonstrated that an inorganic-binding peptide could preferentially better adsorb on to one material (gold) compared to another (silicon dioxide) but also over to one noble metal (gold) compared to another one (platinum). Furthermore, these experiments also demonstrated that a nanoscale hybrid entity, SA-QD, can be directed immobilized over selected regions (Au) on a fairly complex substrate (SiO_2 substrate containing Au and Pt micropatterns) by using an engineered polypeptide as a "molecular glue". The selective and controlled adsorption of inorganic binding peptides may have significant applications in nano- and nanobio-technology where these parameters could potentially be tailored for specific utilization in the development of self-assembled molecular systems.

See, Candan Tamerler, Memed Duman, Ersin Emre Oren, Mustafa Gungormus, Xiaorong Xiong, Turgay Kacar, B. Parviz, M. Sarikaya, "Materials Specificity and Directed Assembly of a Gold Binding Peptide," Small, in print (2007).

g. GEPIs as Molecular Erectors for enhanced Enzyme Immobilization: Immobilization of enzymes is an important phenomenon for proteomics applications, building immumoarrays, enzymatic detection systems. Protein immobilization is mainly done by physical adsorption, chemical entrapment and chemical bonding. Most of these methods yield in a lower activity of the enzyme. A more convenient way using biomolecular interaction is employed in this study. Inorganic binding peptides with their unique recognition capability were employed to attach a model protein streptavidin-alkaline phosphatase (SAAP) fusion on to gold, platinum and silica surfaces. The kinetics of building molecular structures for immobilizing SAAP on gold, platinum and silica surfaces was monitored using surface plasmon resonance spectroscopy (SPR). The activity of the immobilized SAAP was also monitored on SPR slide surface using SPR as monitoring tool. It was observed that immobilization of SAAP via biotin tagged inorganic gold binding peptide (GBP), platinum binding peptide (PtBP) and silica binding peptide (SiOx) enhanced the immobilized enzyme activity.

See, U. Seker et al., JACS, submitted (2007)

h. Assembly Kinetics of Engineered Gold Binding Polypeptide on Gold Using Atomic Force Microscopy: Using an atomic force microscope, we determined the assembly and its kinetics of a genetically engineered gold binding polypeptide on a gold substrate. The peptide is assembled by first nucleating domains which then grow the surface up to 95% coverage density forming a continuous monomolecular thin film. An efficient packing is observed over time as the peptide film is saturated with increased peptide packing density without loosing its monolayer property. Kinetic analysis were performed at two stages; first, over a time scale to establish a period, which will be within the equilibrium of a mid-ranged concentration assembly process, next over a concentration scale at the selected approximate equilibrium time. Langmuir1:1 model reveals a close relationship to observed correlations between coverage and concentration of gold binding peptide on the gold surface. We then compared the results of our kinetics analysis based on *ex-situ* AFM to the ones obtained from *in-situ* SPR. Despite of the differences between two systems, the equilibrium coverage values and equilibrium constants calculated by both systems were found to be within 5% variation and presented 10 fold differences, respectively.

See, Chris So et al., Nanoletters, submitted (2007).

i. Patterned supramolecular assembly of a genetically engineered gold binding protein on Au{111}: The sequence, MHGKTQATSGTIQS, is one of several genetically engineered polypeptides that interact with and assemble onto Au surfaces.[1-5] This polypeptide represents a novel building block for constructing hierarchal assemblies on Au, yet the mechanism and nature of protein – Au binding and assembly are poorly understood. We report novel AFM and molecular simulation studies which detail the formation of ordered supramolecular assemblies of gold binding protein-1 (3R GBP1)[5] on Au {111}. These assemblies possess axial directions that mirror the hexagonal indices of the Au {111} Miller planes. Simulated annealing molecular dynamics (SA/MD) simulations indicate that the lowest energy structure of 3R GBP-1 features periodic regions of extended beta strand and random coil-like regions with surface accessible sidechains for either Au or self-associative interactions. A putative Au docking site was identified on GBP1, and the sidechain atoms of this docking site align with either the <110> or <211> Miller indices of the hexagonal Au {111} surface lattice. Thus, the structure of 3R GBP1 has a number of interesting molecular features that not only explain the observed assembly patterns noted on the Au surface, but can be exploited in future nanotechnology building schemes for constructing periodic protein and nanoscale arrays on Au surfaces.

See, Chris So, et al., in Nature-Materials, submitted (2007).

j. Novel Informatics-Based Knowledge-based Design of Inorganic Binding Peptides: The discovery of peptides that specifically bind to inorganic substrates and their practical application in biotechnology and materials science is accelerating. A greater understanding of the relationships between the peptide sequences and their binding affinities and specificities will enable further design of novel peptides with selected properties of interest. We have developed a bioinformatic approach to classify an unknown peptide according to its inorganic substrate binding properties. Our approach performs all-against-all comparisons of experimentally characterized binders and scores the alignments using established sequence similarity scoring matrices. We then generate new scoring matrices that optimize the self-similarity scores between strong, moderate, and weak binders. Using these scoring matrices, a given new peptide sequence is classified based on similarity to the known experimental binders. We used this approach to classify a set of experimentally characterized quartz binding peptides and computationally designed new quartz binding sequences with specific affinities. Experimental studies using these new computationally designed peptides confirm our predictions with high accuracy. We show

that our approach is generalizable and can be used to design peptides to bind inorganic substrates with predictable affinities and specificities.

See e. E. Oren et al., Nature-Materials and Bioinformatics, Submitted (2007).

k. Molecular Recognition: Metal Recognition of Septapetides via Polypod Molecular Architecture: Structural control of inorganics at the molecular scale is a key to the production of materials with new and improved properties used in a wide range of systems from biomaterials to nanotechnological systems. Biological hard tissues are striking examples that may serve as conceptual models for future biomimetically engineered materials. Hard tissues are biocomposites, i.e., hybrid materials containing biomacromolecules (such as proteins) and bioinorganics (e.g., calcite, magnetite, and silica) resulting in highly functional properties (magnetic, mechanical, and photonic). They have intricate nano-and microarchitectures controlled at the molecular level by macromolecules, often proteins via molecular recognition with high affinity and specificity. Intermolecular interactions between proteins and inorganics are mediated via multiple weak interactions (e.g., electrostatic, polar, hydrogen bonds), which collectively may approach the strength of covalent bonds. It would be desirable to engineer polypeptides with sequences that recognize inorganics with high specificity and to use them as nucleators, growth modifiers, and catalyzers in producing controlled material structures.

*See: E. E. Oren, C. Tamerler, M. Sarikaya, "Metal Recognition of Septapeptides via Polypod Molecular Architecture", Nano Letters, **5** (3), 415-419 (2005), and N. Kantarci, C. Tamerler, M. Sarikaya, T. Haliloglu, P. Doruker, "Molecular Dynamics Simulations on Constrained Metal Binding Peptides", Polymer, **46**, (12) 4307-4313 (2005).*

l. Molecular Recognition - Molecular characterization of a prokaryotic polypeptide sequence that catalyzes Au crystal formation - The gold crystal-forming E. coli polypeptide sequence, MHGKTQATSGTIQS, is one of several polypeptide sequences that interacts with gold interfaces and catalyzes the formation of Au crystals in solution, with nucleated Au crystals preferentially featuring the (111) interface. To date, there have been no experimental studies which explore the structure of E. coli-expressed gold binding proteins or the binding of Au(III) ions by these polypeptides. In this present report, multidisciplinary approaches were applied to the 42-AA gold binding protein-1 (GBP-1/42) and to a model polypeptide representing the 14-AA integral repeat of this protein (GBP1/14). CD and NMR spectroscopy indicate that neither the integral repeat nor the GBP-1 protein adopt folded structures in the apo form or in the presence of Au(III) ions; the integral repeat adopts a random coil-extended structure conformation [i.e.,(MHGKTQA)random coil–(TSGTIQS)extended] and the GBP-1 protein appears to be similarly structured. These features are inconsistent with a templating structure. Mass spectrometry experiments indicate that the integral repeat binds up to two Au(III) ions per polypeptide molecule, and H NMR ROESY experiments pinpoint the interaction of Au(III) within two sites: the -QAT-region of the integral repeat MHGKTQATSGTIQS sequence, and, at the negatively charged C-terminus of this sequence. Collectively, our findings support the hypothesis that GBP-1 does not catalyze Au crystal formation via a templating mechanism; rather, the open, unfolded structure of this protein, combined with the presence of accessible proton donor/acceptor amino acids (Ser, Thr, Lys, Gln, His) most likely play a role in Au crystal formation in solution and may also explain the interactive nature of this polypeptide with Au interfaces.

*See J. L. Kulp, M. Sarikaya, J.S. Evans, "Molecular Characterization of a prokaryotic polypeptide sequence that catalyzes Au crystal formation", J. Mater. Chem., **14** (14), 2325-2332 (2004).*

m. Molecular Recognition - Adsorption of genetically engineered proteins studied by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Part A: data acquisition and principal component analysis (PCA) – Recent progress in the adaptation of combinatorial biology selection protocols to materials science has created a new class of polypeptides with specific affinity to inorganics. Here, we use one of the genetically engineered proteins, a gold binding protein (GBP-1), to assess quantitatively its binding specificity to Au, Ag and Pd surfaces by using time-of-flight secondary ion mass spectroscopy (TOF-SIMS). The GBP-1, originally selected using cell-surface display techniques, consisting of 14 amino acids with a sequence of MHGKTQATSGTIQS, was used in this study. Three-repeat and single-repeat forms of GBP-1 were prepared. In earlier studies, GBP-1 was shown to bind to Au particles and self-assemble on flat Au surfaces. Through the fingerprint analysis of these specific peptides, their role in binding can be investigated in terms of their contribution to surface interaction possibly forming the right molecular architecture for binding. To achieve this purpose, a large-sized data matrix produced by TOF-SIMS must be properly treated for analysis. **Adsorption of genetically engineered proteins studied by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Part B**: hierarchical cluster analysis (HCA): In Part A, we use principal component analysis (PCA) to visualize the spectral variations for a variety of adsorption conditions and suggest possible contribution of the specific types of amino acids (binding site) to the interactions; Adsorption of genetically engineered proteins studied by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Part B: hierarchical cluster analysis (HCA) - In Part A, we adopted principal component analysis (PCA) for the analysis of TOF-SIMS data to assess the binding specificity of GBP-1 to metallic Au, Ag and Pd. Within a given set of data, PCA aids in the interpretation of the TOF-SIMS spectra by capitalizing on the differences from one spectrum to another. In Part B, we introduce another multivariate statistical method called 'hierarchical cluster analysis (HCA)', where visualization of the similarity and difference in data is readily observed, from which a variety of adsorption conditions of GBP-1 were characterized.

See, Noriaki Suzuki, Lara Gamble, Candan Tamerler, Mehmet Sarikaya, David G. Castner, and Fumio S. Ohuchi, "Adsorption of Genetically Engineered Proteins Studied by Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) Part-A: Data Acquisition and Principal Component Analysis (PCA)," Surface and Interface Analysis, 39, 419-426 (2007) and Noriaki Suzuki, Mehmet Sarikaya and Fumio S. Ohuchi Adsorption of Genetically Engineered Proteins Studied by Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) Part-B: Hierarchical Cluster Analysis (HCA), Surface and Interface Analysis 39, 427-439 (2007).

n. Implementation: Directed Enzyme Immobilization Using GEPIs as Molecular Erectors: There is a growing interest in inorganic binding peptides because of their unique molecular recognition and self-assembly characteristics for their ultimate practical applications in nanotechnology. Herein, we demonstrate the utilization of gold binding polypeptide as a molecular linker for the self-directed assembly of alkaline phosphatase on gold surfaces. Cell surface display selected gold binding polypeptide was genetically tailored for the controlled gold surface affinity. Different number of multiple repeats (n= 5, 6, 7) containing gold binding polypeptide constructs were genetically fused to alkaline phosphatase and resulting heterofunctional enzymes were expressed in *E.coli* cells. Heterofunctional and wild type alkaline phosphatase enzymes were purified through ion-exchange and gel filtration chromatography. Phosphatase as well as gold binding activities of hybrid constructs was studied using several biochemical and molecular imaging protocols. We demonstrated the retained activity of the genetically engineered alkaline phosphatase self immobilized on gold surface. The difference in the activity levels of the genetically engineered construct and the wild type are demonstrated the specific and non-specific adsorption behavior of the enzyme on the substrate. Self localizing behavior of the hybrid construct resulted in higher surface density of the enzyme compared to

wild type. Biosorption mechanism caused by the biological recognition of the substrate open up enormous possibilities for novel self-directed assembled immobilization.

See, T. Kacar et al., Submitted to Medical Nanotechnology (2007)

o. Implementation – a. Electrochemical nanofabrication using crystalline S-layers as nanomasks: We have developed a simple and robust method to fabricate nanoarrays of metals and metal oxides over macroscopic substrates using the crystalline surface layer (S-layer) protein of Deinococcus radiodurans as an electrodeposition mask. Substrates are coated by adsorption of the S-layer from a detergent-stabilized aqueous protein extract, producing insulating masks with 2−3 nm diameter solvent-accessible openings to the deposition substrate. The coating process can be controlled to achieve complete or fractional surface coverage. We demonstrate the general applicability of the technique by forming arrays of cuprous oxide (Cu_2O), Ni, Pt, Pd, and Co exhibiting long-range order with the 18 nm hexagonal periodicity of the protein openings. This protein-based approach to electrochemical nanofabrication should permit the creation of a wide variety of two-dimensional inorganic structures.

p. Implementation - Stability of S-layer Proteins for Electrochemical Nanofabrication: Crystalline cell surface layer proteins (S-layers) can be used in electrochemical fabrication to create nanoscale arrays of metals and oxides on surfaces so long as the proteins maintain their long range order during processing. We have explored the stability of the HPI layer protein (the S-layer protein from the microorganism *Deinococcus radiodurans*) adsorbed onto platinum surface after immersion in sulfuric acid or sodium hydroxide electrolytes ranging in pH from 0 to 14 over time periods ranging from 1 to 1000 seconds. Topographic data obtained by atomic force microscopy (AFM) was used to characterize the protein stability, judged by its retention of long range order after immersion. The compiled data revealed that, under these solution conditions and in this environment, the HPI layer protein has a dose-dependent structural stability "envelope" in the acidic range from $1 < pH < 4$. The protein retains its long range order up to 1000 seconds from pH 4 to 11, and has a sharp stability edge between pH 12 and 13. Interestingly, the more stringent requirement of stability (*i.e.*, retention of long-range order) defined in the context of electrochemical fabrication for this protein narrowed the window of stability in pH and time when compared to previous stability studies reported for this protein.

See, D. B. Allred, M. Sarikaya, F. Baneyx, D. T. Schwartz, "Electrochemical Nanofabrication Using Crystalline Protein Masks", Nano Letters, ***5*** *(4), 609-613 (2005), and D. Allred et al., Submitted to Surface Science, accepted (2007).*

q. Implementation - Designer Proteins: Engineered DNA Binding Protein Nonequilibrium Synthesis and Assembly of Hybrid Inorganic-Protein Nanostructures: We show that a protein with no intrinsic inorganic synthesis activity can be endowed with the ability to control the formation of inorganic nanostructures under thermodynamically unfavorable (nonequilibrium) conditions, reproducing a key feature of biological hard-tissue growth and assembly. The nonequilibrium synthesis of Cu_2O nanoparticles is accomplished using an engineered derivative of the DNA-binding protein TraI in a room temperature precursor electrolyte. The functional TraI derivative (TraIi1753::CN225) is engineered to possess a cysteine-constrained 12-residue Cu_2O binding sequence, designated CN225, that is inserted into a permissive site in TraI. When TraIi1753::CN225 is included in the precursor electrolyte, stable CuO nanoparticles form, even though the concentrations of [Cu] and [OH] are at 5% of the solubility product ($K_{sp,Cu2O}$). Negative control experiments verify that Cu_2O formation is controlled by inclusion of the CN225 binding sequence. Transmission electron microscopy and electron diffraction reveals a core–shell structure for the nonequilibrium nanoparticles: a 2 nm Cu_2O core is surrounded by an adsorbed

protein shell. Quantitative protein adsorption studies show that the unexpected stability of Cu_2O is imparted by the nanomolar surface binding affinity of TraIi1753::CN225 for Cu2O (K_d=1.2x10-8 M), which provides favorable interfacial energetics (–45 kJ/mol) for the core-shell configuration. The protein shell retains the DNA-binding traits of TraI, as evidenced by the spontaneous organization of nanoparticles onto circular double stranded DNA.

*See, H. X. Dai et al., nanoequilibrium synthesis and assembly of hybrid inorganic-protein nanostructures using an engineered DNA binding protein, J. Amer. Chem. Soc., **127** (44) 15637-43 (2005).*

r. Implementation: Hybrid Molecular Constructs for Functionality: Proteins initiate, catalyze and mediate the fabrication of nano-and microstructures, which are then assembled into complex architectures necessary for specific biological functions. Biomimetic systems could include proteins to control not only the synthesis but also the spatial distribution of inorganic materials into functional assemblies with useful electrical and optical properties. There have been reports on the assembly of nanoparticles in solution and the directed immobilization of nanoparticles on substrates using protein based recognition systems. Over the last decade, the use of display technologies has emerged as a powerful strategy to screen peptides with recognition for the surface of an inorganic material. In pioneering efforts, we have explored the patterning of genetically engineered polypeptides by microcontact printing using GBP-1.While it is possible, forced immobilization of proteins on the surface may lead to the loss of recognition or self assembly characteristics. Spatial conformation has a profound effect on the ability of GBP-1 in guiding the assembly of gold nanoparticles.

*See, for example, M. T. Zin, H. Ma, M. Sarikaya and A. Jen, "Assembly of gold nanoparticles using genetically engineered polypeptides," SMALL, **1**(7) 698-702 (2005).*

s. Implementation – Well-Controlled Arrays of Core-Shell Quantum Dots with Tunable Photoluminescence Properties - We report two studies that aim to modulate the photoluminescence (PL) properties from arrays of quantum dots (QDs) by controlling the spatial organization of QDs with respect to the underlying metal (Au or Ag) surface. The first study explores the use of polypeptides and organic-peptide conjugates as two linkers with well-defined heights to attach QDs at known separations to the metal surface. This approach demonstrates a molecular biomimetic approach toward nanophotonics by integrating inorganic, organic, and biomolecular constructs to form hybrid nanostructures through template-directed self-assembly. In the second study, QDs are attached onto arrays of metal nanostructures. This approach affords the tailoring of localized surface plasmon resonance (LSPR) spectra of metal nanostructures as well as the tuning of vertical separation of QDs from the metal surface through layer-by-layer self-assembly.

*See, M. T. Zin, H. Ma, M. S. Kang, S. H. Kang, K. S. Kim, M. H. Zareie, K. Leong, M. Sarikaya, and A. K.-Y. Jen, "Controlled Self-Assembly of Nanomaterials through Highly Ordered Self-Assembled Monolayers and Genetically Engineered Polypeptides", Poly. Mater. Sci. Eng., **95**, 1071 (2006).*

t. Peptide-mediated surface-immobilized quantum dot hybrid nanoassemblies with controlled photoluminescence - Combinatorially selected peptides and peptide–organic conjugates were used as linkers with controlled structural and organizational conformations to attach quantum dots (QDs) at addressable distances from a metal surface. This study demonstrates an approach towards nanophotonics by integrating inorganic, organic, and biological constructs to form hybrid nanoassemblies through template-directed self-assembly.

Peptide–organic-linked QD arrays showed stronger fluorescence than peptide-linked QD arrays. We attribute this difference primarily to the increased number density of QDs on peptide–organic-linked QD arrays.

See, for example, M. T. Zin, A. M. Munro, M. Gungormus, N. Y. Wong, H. Ma, C. Tamerler, D. S. Ginger, M. Sarikaya and A. K. Y, Jen "Peptide-mediated surface-immobilized quantum dot hybrid nanoassemblies with controlled photoluminescense", J. Materials Chemistry*, **17**, 866-872 (2007).*

Implications of the Research & Conclusions: The **overarching goal** of this project has been to create conditions under which GEPI molecules can be used for assembling nanoscale materials for practical technological applications. As sampled above, to reach this goal, we use molecular biomimetics principles, which provide means to overcome the difficulties that limit the current progress of nanotechnology, i.e., non-specific recognition, random (stochastic) distribution and order, synthetic microfabrication, and chemical design. In molecular biomimetics, hybrid materials could potentially be assembled from the molecular level using the recognition properties of proteins under the premise that inorganic surface-specific polypeptides could be used as binding agents to control the organization and specific functions of materials. Molecular biomimetics simultaneously offers three solutions to the development of hetero-functional nanostructures. The *first* is that protein templates are designed at the molecular level through genetics. This ensures molecular processing for nanostructural control at the lowest dimensional scale possible (i.e., DNA-based). The *second* is that surface-specific proteins can be used as linkers to bind synthetic entities, including nanoparticles, functional polymers, or other nanostructures onto molecular templates (molecular and nanoscale recognition). Finally, the *third* solution harnesses the ability of biological molecules to self- and co-assemble into ordered nano-structures. This ensures a robust assembly process for achieving complex nano-, and possibly hierarchical-structures, similar to those found in Nature (self-assembly). The examples given above are but a portion of the research that has been accomplished within this period of the DURINT project attesting the accomplishments towards the goals set in the proposal.

5. POTENTIAL IMPACT OF THE PROPOSED RESEARCH ON DoD INTERESTS: SIGNATURE CONTROL: Signature control in DoD systems has been of great importance, especially with the advent of more accurate weapons, and greater ease of target acquisition. The detection of identification of tanks, aircraft, ships, land mines, and enemy personnel have received much attention during the past decade, the latter in the context of clandestine urban warfare. The other side of the coin has to do with evading such detection, and more-and-more sophisticated means have been designed to carry out such evasion. Of particular importance is the ability to tune evasion (and detection) techniques over a wide range of conditions (wavelengths, temperatures, etc.). Also, there are active and passive methods, of both detection and evasion in the signature control area, and man advances have been made in both remote and proximal multispectral method of detection.

The areas of Intelligence of Electronic Warfare, and Engineer and Mine Warfare are two major ARMY areas that are potential beneficiaries of the results of the proposed research. First, the enormous potential impact of molecular switching for nanoscale electronic devices can be seen from the extreme miniaturization that could become feasible as part of the proposed work (on functional polymer/protein/inorganic hybrid structures and their nanoassembly). The other example is that of bioinspired pathways which incorporate quantum dots (QD) of a wide range of sizes for infrared sensing, detection, and imaging. Applications in mine detection, and detection of intrusions, would be of immediate interest. In addition to the foregoing, many other applications in electronics and photonics, employing multispectral arrays, photovoltaics, and

other devices, for displays employed in a number of platforms, and both tactical and strategic applications, can be envisioned.

In this final report of this UW-DURINT supported by ARO, we tried to give a full account of the results in the formal marriage of materials sciences and engineering and genetics/molecular biology in the genetic selection and design of inorganic binding peptides, understanding the mechanisms of peptide-inorganic interactions, and the utility of these peptides as molecular erectors, assemblers, and synthesizers as fundamental blocks in practical technology and medicine, outlined as the new paradigm of Molecular Biomimetic, a new field emerged from this DURINT project at the confluence of materials, biology, and genetics, which encompasses nanotechnology, bionanotechnology, molecular engineering, and molecular medicine.

REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

1. Papers published in peer-reviewed journals

1. C. Tamerler and M. Sarikaya, "Molecular biomimetics: Utilizing Nature's molecular ways in practical engineering", *Acta Biomaterialia*, **3**, 289-299 (2007); Review.
2. C. Tamerler, T. Kacar, D. Sahin, H. Fong and M. Sarikaya, "Genetically engineered polypeptides for inorganics: A utility in biological materials science and engineering", *Materials Science and Engineering C*, **27**, 558-564, (2007).
3. D. B. Allred, M. T. Zin, H. Ma, M. Sarikaya, F. Baneyx, A. K. Jen and D. T. Schwartz, "Direct nanofabrication and transmission electron microscopy on a suite of easy-to-prepare ultrathin film substrates", *Thin Solid Films*, **515**, 5341-5347, (2007).
4. A. Presenda, D. B. Allred, F. Baneyx, D. T. Schwartz, and M. Sarikaya, "Stability of S-layer proteins for electrochemical nanofabrication," *Colloids and Surfaces-B*, in print (2007).
5. Noriaki Suzuki, Lara Gamble, Candan Tamerler, Mehmet Sarikaya, David G. Castner, and Fumio S. Ohuchi, "Adsorption of Genetically Engineered Proteins Studied by Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) Part-A: Data Acquisition and Principal Component Analysis (PCA)," *Surface and Interface Analysis,* **39,** 419-426 (2007).
6. Noriaki Suzuki, Mehmet Sarikaya and Fumio S. Ohuchi Adsorption of Genetically Engineered Proteins Studied by Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) Part-B: Hierarchical Cluster Analysis (HCA), *Surface and Interface Analysis* **39**, 427-439 (2007).
7. M. T. Zin, H. Ma, A. M. Munro, D. S. Ginger, M. Gungormus, K. Leong, C. Tamerler, M. Sarikaya and A. K. Jen, "Well-controlled arrays of core-shell quantum dots with tunable photoluminescence properties", *Poly. Mater. Sci. Eng.*, **95**, 1002, (2006).
8. M. T. Zin, A. M. Munro, M. Gungormus, N. Y. Wong, H. Ma, C. Tamerler, D. S. Ginger, M. Sarikaya and A. K. Y, Jen, "Peptide-mediated surface-immobilized quantum dot hybrid nanoassemblies with controlled photoluminescense", *Journal of Materials Chemistry*, **17**, 866-872 (2006).
9. D. T. Schwartz, "Electrodeposition and Nanobiosystems," *Interface Mag*. **15** (1), 34-35 (2006).
10. C. Tamerler, M. Duman, E. E. Oren, M. Gungormus, X, Xiong, T. Kacar, B. A. Parviz and M. Sarikaya, "Materials Specificity and Directed Assembly of a Gold-Binding Peptide", *Small*, **2**, 1372-1378 (2006).
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12. E. E. Oren, C. Tamerler, M. Sarikaya, "Metal Recognition of Septapeptides via Polypod Molecular Architecture", *Nano Letters*, **5** (3), 415-419 (2005).
13. H. Dai, W.-S. Choe, C. K. Thai, M. Sarikaya, B. A. Traxler, F. Baneyx, D. T. Schwartz, "Nonequilibrium Synthesis and Assembly of Hybrid Inorganic-Protein Nanostructures Using an Engineered DNA Binding Protein", *J. Am. Chem. Soc.*, **127** (44), 15637-15643 (2005).
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20. S. N. White et al., "Controlled failure mechanismss toughen the dentino enamel junction zone," Jrl. Of Prothetic Dentistry, **94**, 40 330-335 (2005).
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26. E. DiMasi and M. Sarikaya, "Synchrotron x-ray microbeam diffraction from abalone shell", *J. Mater. Res.*, **19** (5), 1471-1476 (2004).
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2. Non-peer reviewed papers

None

3. Presentations (all invited)

Sarikaya and colleagues presented, through this DURINT project, 150 invited talks and 200 contributed presentations (poster and platform) in many workshops, such as Molecular Biomimetics, (Friday Harbor lab, San Juan Island, WA), Foundations of Nanoscience (Snowbird), Biomimetic Processing (Nagoya, Japan), Bionanotechnology (Istanbul, Turkey), and many conferences (e.g., Fall and Spring Meetings of MRS, Annual Meetings of American Chemical Society, American Physical Society, American Society of Microbiology, Colloid Science, and Crystallization, plus Gordon Conferences, etc.). Some of the Invited Presentations are as follows (chronologically):

1. Sarikaya, M., *American Chemical Society*, 234th Meeting, Boston, MA, August 19-23, 2007.
2. Sarikaya, M. "Crystal Morphogenesis using Engineered Peptides," *International conference on Crystal Growth*," Salt Lake City, August 12-17, 2007.
3. Sarikaya, M., "Kinetics and Thermodynamics of Peptide-Solid Interactions," in *Modeling and Interactions of Biomolecules with Inorganic Surfaces*," Paris, France, July 24-27, 2007.
4. Sarikaya, M., "Genetically Engineered peptides for Nanotechnology and Medicine," *in 2nd International Workshop on Bionanotechnology*, Istanbul, Turkey, May 23-25, 2007.
5. Sarikaya, M., "Genetically Engineered peptides for Nanotechnology and Medicine," *Materials Days, University of Rostock*, Germany, May 3-4, 2007.
6. Sarikaya, M. "Genetically Engineered Materials Using Peptides," at *Materials Research Science and Engineering Center, University Wisconsin*, Madison, WI, April 24, 2007.

7. Sarikaya, M., Genetically Engineered Peptides for Technology and Medicine, in *Symp. Peptide-Based Biotechnology, Annual Meeting of the Society of Biomaterials*, Chicago, April 18-21, 2007.
8. Samudrala, R., "In Silicon Design of Inorganic-Binding Peptides," *4th annual Conference on Foundations of Nanoscience*, FNANO07, Snowbird, UTAH, April 18-21, 2007.
9. Tamerler, C., Genetically Engineered peptides for Nano- and Bionanotechnology," *4th annual Conference on Foundations of Nanoscience*, FNANO07, Snowbird, UTAH, April 18-21, 2007.
10. Sarikaya, M. "Genome-based Materials Science," in Exploring and Exploiting Nature with Biomimetics, *American Chemical Society*, Chicago, IL, March 27, 2007.
11. Sarikaya, M., Molecular Biomimetics and Bionantotechnology for Medicine, *2nd International Conference on Molecular Medicine*, Istanbul, Turkey, March 22-26, 2007.
12. Sarikaya, M., Molecular Biomimetics and GEMSEC, *CNT Seminar Series*, Center for Nanotechnology, University of Washington, Seattle, March 13, 2007.
13. Tamerler, C., and Sarikaya, M., Post-Selection Engineering of Inorganic-Binding Peptides: Tools for Bionanotechnology, *TMS Annual Meeting*, Orlando, FL., February 27, 2007.
14. Sarikaya, M., Biomimetics and Bioinspired Materials, *TMS Annual Meeting*, Orlando, FL., February 27, 2007.
15. Sarikaya, M. Molecular Biomimetics Series of Presentations, at *Ecotopia Science Institute*, Nagoya University, Japan, January 12, 2007.
16. Sarikaya, M., Metrology Using Engineered Peptides, in *Metrology Workshop, Intel* Sponsored, San Francisco, CA, December 14-15, 2006.
17. Sarikaya, M., *2nd International Conference on Biomechanics*, Istanbul Technical University, Istanbul, Turkey, December 1-3, 2006.
18. 6 presentations by the members of the DURINT project at *Biomimetics-I: Annual Workshop on Molecular Biomimetics*; speakers; Tamerler, C., Evans, J., Baneyx, F., Traxler, B., Jen, A., and Sarikaya, M., Friday Harbor Marine Lab., San Juan Island, WA, September 6-8, 2006.
19. Tamerler, C., "Genetic Engineering Tools on Creating Materials" *GORDON Conferance on Biomineralization*, Colby-Sawyer College, New London, NH, August 1-4, 2006.
20. Sarikaya, M., "Protein-based Materials," *Hacettepe University Medical School*, June 27, 2006.
21. Sarikaya M., 'Molecular biomimetics: nanotechnology through Biology," *3rd European Conference on Regenerative Medicine*, Rostock, Germany, June 19-25, 2006.
22. Sarikaya, M., "Molecular Biomimetics: protein-based materials for ecological Living," Review of *Ecotopia Science Institute*, Nagoya University, Japan, June 13-14, 2006.
23. Sarikaya, M., "Molecular Biomimetics," *Yale University*, New Haven, CN, May 22, 2006.
24. Sarikaya, M. "Molecular Biomimetics," *Boston University*, Boston, MA, May 13, 2006.
25. Schwartz, D. T. "BioCAM: Biology and computer aided manufacturing" *Colloquium seminar at UC Riverside*, April, 2006.
26. Tamerler, C., "Self Assembled Peptide Based Functional Molecular Constructs" *3rd Annual Meeting of Foundation of Nanoscience*, Snowbird, Utah, April 24-27, 2006.
27. Schwartz, D., *Cambrios Technology*, "Nonequilibrium synthesis of inorganics using engineering proteins." March 2006.
28. Tamerler, C., "Designing Heterofunctional Proteins for Functional Materials and Systems" *TMS Symposium on "Biological Materials Science and Engineering"*, San Antonio, USA, March, 2006.
29. Sarikaya, M. "Molecular Biomimetics – Peptide Based Materials," MSE Dept. and MRSEC, *Northwestern University*, Evanston, IL, February 2006.
30. Sarikaya, M. *135th Ann. Meeting of TMS* (The Metals, Minerals and Materials Society), San Antonio; February 2006.
31. Sarikaya, M. "Biomimetics – Peptide Based Materials," BioDesign Institute, *Arizona State University*, Tempe, AZ, February 2006.

32. M. Sarikaya, "Synthetic Biology: Protein-based Materials Science and Engineering," *BMMP-5; 5th International Symposium on Biomimetic Materials Processing*, January 26-28, 2006, Nagoya, Japan.
33. Sarikaya, M. "Molecular Biomimetics: Materials Science and Engineering through Biology," Genome Research Center, *Academia Sinica, Taipei*, Taiwan, January 2006.
34. Baneyx, F. "Molecular biomimetics: protein-aided nanofabrication". *Pacific Rim Summit on Industrial Biotechnology and Bioenergy*, Honolulu, HI, January 2006.
35. Tamerler, C., "Selection Engineering of inorganic binding peptides for improved functionality", *Sixth International Symposium on Biomimetic Materials Processing (BMMP-5)*, January, 2006, Nagoya, Japan.
36. Baneyx, F. "Protein renaturation and refolding". *NYAS/FDA/NIST Follow-on Biologics Workshop*, New York, NY, December 2005.
37. Sarikaya, M. and Tamerler, C., "Molecular biomimetics for for soldier systems," *Natick Soldier System Lab., ARL, Nattick*, MA; December 8, 2005.
38. Sarikaya, M. "Molecular Biomimetics," at *Biomimetics and Bionanotechnology Conference*, University of Sydney, Sydney, Australia, December 5-10, 2005.
39. Sarikaya, M., Two invited talks at the *Fall Meeting of MRS*, November 28-December 3, 2005.
40. Sarikaya, M., *A Molecular biomimetics presentation at MRSEC Directors' Meeting*, Arlington, VA, November 2005.
41. Baneyx, F. "An overview of nanotechnology". *American Association of Blood Banks Annual Meeting*, Seattle, WA, October 2005.
42. Sarikaya, M.*, at Biological Materials and biotechnology, Joint Meeting of ASM, ASME, and A CER Soc.*, Philedelphia, PA, October 2005.
43. Sarikaya, M., *SPIE, Optics-East*, Boston, PA, October 2005.
44. M. Sarikaya, "Molecular Biomimetics and Nanotechnology" presented at the Science Meets Engineering, at TUBITAK, *Turkish Scientific and Technology Institute*, Ankara, TURKEY, October 2005.
45. Baneyx, F. "Nanotechnology and its potential applications in biology and life sciences". *Nanotechnology User Facility Characterization and Fabrication at the Nanoscale Short Course*, Seattle, WA, September 2005.
46. Sarikaya, M., *1st Conference by Ecotopia Science Institute*, International Invited speaker at the, Nagoya, Japan, August 2005.
47. Sarikaya, M., *American Society of Crystallography and Crystal Growth*, Big Sky, Montana, July 2005.
48. Sarikaya, M. *et al.*, "Engineered inorganic-binding polypeptides for functional nanoinorganics," *Naval Research Laboratory, Nanotechnology Center*, Washington, D.C., June 2005.
49. Sarikaya, M. *Conference on Colloids* organized by American Chemical Society, Clarkson Univ., Up State New York, June 2005.
50. Sarikaya, M. "Molecular Biomimetics: *Workshop on Biotechnology*, MOBGAM, Istanbul Technical University, Istanbul, Turkey, May 2005.
51. Sarikaya*,* M., *1st Conference on Nanotechnology,* Bilkent University, Ankara, Turkey, May 2005.
52. Sarikaya, M., "Molecular Biomimetics: Progress Report," Center for Molecular Biology and Genetics, *MOBGAM, Istanbul Technical University*, Istanbul, Turkey, March 2005.
53. Sarikaya, M., "Molecular Biomimetics: Progress Report," Chemistry Department, *Hacettepe University*, Istanbul, Turkey, March 2005.
54. Sarikaya, M.*, 135th Ann. Meeting of TMS* (The Metals, Minerals and Materials Society), San Antonio; February 2005.

55. Sarikaya, M., *Materials Research Society 2004 Fall Meeting*, Boston, Massachusetts, November 29-December 3, 2004. "Biomimetic Lessons: From Mother-of-Pearl to Mammalian Dental Tissues Materials."
56. Sarikaya, M., *Materials Research Society 2004 Fall Meeting*, Boston, Massachusetts, November 29-December 3, 2004. "Structural Biomimetics: Mechanical design of materials through biology".
57. Sarikaya, M., *SPIE, Optics-East*, Philadelphia, PA, October 22-25, 2004. "Materials assembly using engineered polypeptides".
58. Baneyx, F., Thai, C.K., Dai, H., Sastry, M.S.R., Sarikaya, M., Schwartz, D.T., *UW Nanoscale Science and Technology Workshop*, Seattle, WA, September, 2004. "Identification and characterization of Cu_2O, and ZnO binding polypeptides by Escherichia coli cell surface display".
59. Schwartz, D.T., Dai, H., Choe, W.S., Thai, C.K., Traxler, B.A., Baneyx, F., *UW Nanoscale Science and Technology Workshop*, Seattle, WA, September 2004. "Synthesis and organization of non-equilibrium nanoparticles".
60. Suzuki, N., Gamble, L., Castner, D., Sarikaya, M., and Ohuchi, F., *UW Center for Nanotechnology / Pacific Northwest National Laboratories Symposium*, Seattle, WA, September 16-17, 2004. "Analysis of Short Peptides Adsorption on Metals by Time-of-Flight Secondary Ion Mass Spectroscopy".
61. Sarikaya, M., *International Workshop: Biomimetics-III*, Nagoya University, Nagoya, Japan, January 27-29, 2003; "Self-assembled Nanostructures using genetically engineered polypeptides."
62. Sarikaya, M., *Materials Research Society Fall Meeting*, Boston, MA, December 2-6, 2002, "Molecular Biomimetics: Nanoassembly of materials through biology."
63. Sarikaya, M., *ASM-TMS Fall Meeting*, American Society of Materials-International and The Metals, Minerals and Materials Society Joint Meeting, Columbus, OH; November 4-10, 2002; "Biomimetics: Structure-Property Correlation in Hard Tissues – Lessons from Biology."
64. Sarikaya, M., *Pacific Coast Regional Meeting of the American Ceramic Society*, Seattle, WA, October 12-16, 2002; "Molecular biomimetics: Materials design via biology."
65. Sarikaya, M., *Biomimetics-III*, 3rd Intl, Topical Workshop, titled *Nature of the organic/inorganic interfaces*; Friday Harbor Laboratory, San Juan Island, WA, August 27-29, 2002; "Molecular Biomimetics."
66. Sarikaya, M., AFOSR Workshop on *Nanoscale Approaches to Multifunctional Coatings*, Keystone, CO, August 12-17, 2002; "Molecular Biomimetic approach to protein engineering for inorganics for functional coatings.
67. Sarikaya, M., et al. International Society of Optical Engineering (SPIE) *47th Annual Meeting, Symposium: Properties of Metal Nanostructures*, Seattle, WA; July 7-11, 2002. "Assembly of nanoparticles via engineered polypeptides as molecular linkers."
68. Sarikaya, M., *7th International Materials Conference*, Istanbul, Turkey, June 4-6, 2002; "Biomimetics: Materials Synthesis through Biology," Keynote Presentation.
69. Sarikaya, M., American Society of Civil Engineers (ASCE) - *Symposium on Mechanics of Biological Materials*, Columbia University, New York, June 2-4, 2002; "Mechanical Design of Biological Hard Tissues."
70. Sarikaya, M., and Jen, A., AFOSR Topical workshop on: *Bioinspired Technologies*, Washington, D.C., April 30-May 1, 2002; "Biomimetic pathways to functional nanostructures."
71. Sarikaya, M., International Workshop: *Biomimetic Engineering*, Knowledge Foundation, Orlando, Florida; March, 16, 2002; Keynote Presentation: "Molecular biomimetics: nanoassembly of materials through biology."

72. Sarikaya, M., *International Symposium on Biointegrated Materials & Tissue Engineering*, Japan Science and Technology Corporation, Tokyo, Japan; March 7-8, 2002; "Molecular Biomimetics."
73. Sarikaya, M., *2nd Intl. Conf. on Biomimetics*, Nagoya, Japan; January 10-12, 2002; Plenary Presentation: "Molecular biomimetics."
74. Sarikaya, M., *International Conference on: Soft-Solution Processing of Materials*, Japan Society of Ceramics, Yokohama, Japan, December 11-15, 2001;"Soft solution processing via biomimetics."
75. Sarikaya, M., *Materials Research Society Fall Meeting*, Boston, MA, December 2-6, 2001; "Biomimetics: nanoassembly using engineered polypeptides."
76. Sarikaya, M., *Bioengineering Topical Conference*, ASME (American Society of Mechanical Engineers), San Diego, CA; June 28-30, 2001;"Biomimetic approaches to bioengineering."
77. Sarikaya, M., *Engineering Mechanics*, ASME (American Society of Mechanical Engineers), Orlando, FL, June 3-5, 2001; "Biomimetics: structure-mechanical property correlations in biological hard tissues."
78. Sarikaya, M., *American Chemical Society Spring Meeting*, San Diego, April 12-16, 2001; "Biomimetic strategies in nanomaterials design and engineering."

4. Peer-reviewed Proceedings

1. C. Tamerler, B. Parviz, and M. Sarikaya, "Self-assembled peptide-Based Functional Molecular Constructs," in *Fundamentals of Nanoscience*, J. Reiff (ed.) (Science Technical, Inc., New York, 2006), pp. 181-184.

5. Non-peer reviewed publications (conference proceedings) - None

Below is a Partial List of Citations in Popular Scientific Journals, Trade Journals, Television Programs, Interviews with International Students - Sarikaya's work is also cited at various media in 2004/2005, some examples are (see others in google, msn.com, yahoo):

Google increasingly gives thousands of ~"Molecular Biomimetics" hits; some examples:

- http://www.extra.reading.ac.uk/eng/BIONIS/current_issues.htm
- http://nano.cancer.gov/news_center/monthly_feature_2005_jul.asp
- http://www.esi-topics.com/fbp/fbp-december2004.html
- http://www.nsf.gov/news/news_summ.jsp?cntn_id=104462&org=NSF&from=news
- http://www.horizonpress.com/hsp/abs/absnano.html
- http://72.14.203.104/search?q=cache:04iEpWrexUIJ:www.biotec.boku.ac.at/fileadmin/_/H792nano/docs/Phagedisplay.pdf+MOLECULAR+BIOMIMETICS&hl=en&gl=us&ct=clnk&cd=21
- http://www.extra.reading.ac.uk/eng/BIONIS/current_issues.htm
- http://www.bu.edu/dbin/ece/web/news/item.php?item_id=278
- http://www.dodsbir.net/sitis/archives_display_topic.asp?Bookmark=10941http://www.stormingmedia.us/11/1159/A115914.html
- http://www.lifeedu.org/window_studyguide10.html
- http://72.14.203.104/search?q=cache:Tn5e2YKMyL0J:www.onr.navy.mil/about/conferences/rd_partner/2005/docs/past/2005/0507_guard_human_systems.pdf+MOLECULAR+BIOMIMETICS&hl=en&gl=us&ct=clnk&cd=55
- http://www.wiley-vch.de/publish/dt/books/ISBN3-527-31115-7/
- http://www.powells.com/cgi-bin/biblio?inkey=2-0300105061-1

- ISI Essential Science Indicators, Fast Breaking Papers, http://www.esi-topics.com/fbp/fbp-december2004.html
- ***NanoScout*** – Portal for the Nanotechnology Community – Nanotechnology Research Groups around the world, Sarikaya's DURINT web-site is one of 80 highly cited web-sites: http://www.nanoscout.de/groups.php?sort=country&order=ASC.
- Masive Change, Interviews with Janine Banyus; http://www.massivechange.com/JanineBenyus.html
- etc..

6. Manuscripts submitted but not published (2007)

1. C. So, C., H. Fong, U. Seker, C. Tamerler, and M. Sarikaya, "Adsorption kinetics of genetically engineered gold binding using atomic force microscopy," *Nanoletters*, submitted (2007).
2. T. Kacar, C. So, C. Tamerler, and M. Sarikaya, "Directed enzyme immobilization using a gold binding protein as an erector," *Biotechnology and Applied Biochemistry*, (2006).
3. U. Seker, B. Wilson, D. Sahin, C. Tamerler, and M. Sarikaya, "Cross-specificity of inorganic binding peptides," *Langmuir,* accepted (2007).
4. E. E. Oren, R. Samudrala, C. Tamerler, D. Sahin, S. Dincer, and M. Sarikaya,"Similarity analysis of peptides generated via directed evolution," submitted to *Nature-Materials* (2007).
5. T. Kacar, M. T. Zin, B. Wilson, S. Sepehri, A. Jen, H. Ma, C. Tamerler, and M. Sarikaya, "Template-directed self immobilization of Alkaline phosphotase on Micropatterened surfaces via genetically fused metal-binding peptide, *Small*, submitted (2007).
6. E. E. Oren, C. Tamerler, D. Sahin, N. Karaguler, M. Hnilova, M. Sarikaya, and R. Samudrala, "Bioinformatics design of inorganic binding peptides," *Bioinformatics*, submitted (2007).
7. C. So, J. Kulp, III, E. E. Oren, C. Tamerler, J. Evans and M. Sarikaya, "Patterened Macromolecular assembly of an engineered peptides on inorganic surface," Submitted to *Nature-Biotechnology* (2007).
8. B. Wilson, U. Seker, C. Tamerler, and M. Sarikaya, "The utility of Engineered Polypeptides for Molecular erectors," Submitted to *PNAS* (2007).
9. U. Seker, B. Wilson, C. Tamerler, M. Sarikaya, "Quantitative affinity of genetically engineered inorganic binding peptides by surface Plasmon Resonance Spectroscopy" submitted to *Langmuir* (2007).
10. U. Seker, B. Wilson, C. Tamerler, and M. Sarikaya, "Thermodynamic properties of an Engineered gold Binding Protein on gold," *JACS*, Submitted (2007).
11. M. T. Zin, K. Leong, N. Y. Wong, H. Ma, M. Sarikaya, and A. K.-Y. Jen, "Biomolecular Recognition-Mediated Fabrication of Tunable Quantum Dot Arrays with Surface-Plasmon-Enhanced Fluorescence", Nano Lett. 2007, to be submitted.
12. H. Ma, M. T. Zin, M. H. Zareie, M-S. Kang, S. H. Kang, K. S. Kim, B. W. Reed, M. Sarikaya, and A. K-Y. Jen, "Assembly of Nanomaterials through Highly Ordered Self-Assembled Monolayers and Peptide-Organic Hybrid Conjugates as Templates", *J. Nanosci. & Nanotech.*, (in press).

7. Number of Books

1. C. Tamerler and M. Sarikaya, *Molecular Biomimetics: Linking peptides with inorganic structures* in "Microbial Bionanotechnology: Biological Self-assembly Systems and Biopolymer-based Nanostructures", Ed: Bernd Rehm, Chapter 8, pp: 191-221 (Horizon, London, 2006).

2. C. Tamerler and M. Sarikaya, *Molecular Biomimetics: Building Materials Nature's Way, One Molecule at a Time* in "Nanofabrication Towards Biomedical Applications: Techniques, Tools, Applications, and Impact", Eds: C.S.S.R. Kumar, J. Hormes, C. Leuschner, pp. 119-134 (Weinheim, Germany, 2005).

8. Honors and Awards

- Alex Jen, SPIE Fellow, The International Society of Optical Engineering, 2005.
- Alex Jen, AAAS Fellow, American Association for the Advancement of Science, 2005.
- Francois Baneyx, Charles WH Matthaei Professor of Chemical Engineering and Interim Director of University of Washington Center for Nanotechnology.
- Schwartz, D., Associate Dean for Infrastructure, College of Engineering, University of Washington
- Alex Jen, Interim Chair, MSE Dept., UW
- M. Sarikaya, Institute Professor at Ecotopia Science Institute, Nagoya University, Nagoya, Japan
- M. Sarikaya, awarded a Materials Research Science and Engineering Center by National Science Foundation, based on the research developed in UW-DURINT project discussed herein.; One of the two MRSECs awarded in 2005.

9. Scientific Progress and Accomplishments

See below (extended summary)

10. Number of Patents - One

11. List of Patents - One

Title of the Patent: “Unsupported, electron transparent films and related methods;” The invention relates to unsupported, electron transparent films and methods for making and using unsupported, electron transparent films. The Authors: Daniel B. Allred and Daniel T. Schwartz

12. Technology Transfer

Companies in Contact (monitoring our research progress for marketable products)

- Echobio, Bainbridge Island, WA (Ken Perry)
- Sienna Technologies, Inc., Woodinville, WA (ender Savrun)
- Boston Scientific Co., Redmond, WA (
- Battelle Columbus, Ohio (
- Synthetech, Inc., Oregon (Paul Ahrens)
- Biohesion, Seattle, WA
- Nanoink, Chicago, IL.

Government Laboratories with which we are in collaboration:

- Army Research Lab - Soldier Systems (Charlene Mello), Natick, MA;
- Air Force Research Lab., Wright-Pat Air Force Base, OH (Morley Stone);
- Brookhaven National Lab, New York (Eleine DiMasi)
- Lawrence Livermore National Laboratory, Livermore, CA (Bryan Reed)
- Pacific Northwest National Lab., Richland, WA (Eric Ackerman and Jun Liu)

13. Graduate Students (17)

- Turgay Kacar (M), Grad Student
- Urartu O.S. Seker (M), Grad Student
- Arzu Ozmus (F), Grad Student
- Deniz Sahin (M), Grad Student
- Sibel Cetinel (F), Grad Student
- Hilal Yazici (F), Grad Student
- Mustafa Gungormus (M), Grad Student
- Brandon Wilson (M), Grad Student
- Martin Galan (M) Grad Student
- Melvin Zin (M), Grad Student
- Saghar Sepehri (F), Grad Student
- Haixia Dai (F), Grad Student
- Daniel Allred (M) Grad Student
- Lindsay Kato (F) Grad Student
- Rosalia Tunguska (F), Grad Student
- John Kulp, III (M)(NYU), Grad Student
- Noriyaki Suzuki (M), MSE, Grad Student

14. Faculty (8)

- M. Sarikaya, PI, Materials Science and Engineering and Chem. Eng. (0.15)
- Francois Baneyx, Co-PI, Chemical Engineering (0.1)
- John Evans, Chemistry, New York University (0.05)
- Fumio Ohuchi, MSE (0.05)
- Daniel Schwartz, Chemical Eng. and MSE (0.05)
- Alex Jen, MS, and Chemistry (0.05)
- Candan Tamerler, MSE and Molecular Biology (0.4)
- Beth Traxler, Microbiology (0.15)

COLLABORATORS:

- Ram Samudrala, Microbiology, University of Washington
- Rene Overney, Chemical Engineering, University of Washington
- David Ginger, Chemistry, University of Washington
- Glen Bartholomew, Chemistry, University of Washington
- Stanley Brown, Molecular Biology, University of Copenhagen, Denmark
- Klaus Schulten, Biophysics, University of Illinois, Urbana-Champaign
- Charlene Mello, Natick Soldier System Laboratory, Natick, MA
- Jonathan Trent, NASA Ames, San Jose, CA
- Morley Stone, Air Force Research Laboratory, Dayton, OH
- Eric Ackerman, Pacific Northwest National Laboratory, Richland, WA
- Joel Schneider, Chemical Eng., University of Delaware, Newark, DE.

15. Post-doctoral researchers (11 - partial)

- Marketa Hnilova (MSE) (full – 1 year)
- Hadi Zareie (MSE) (full – 2 years)
- Brian W. Reed (MSE) (full – 2 years)
- Hanson Fong (MSE) (10% – 3 years)
- Emre Oren (MSE and Microbiology) (half – 3 years)
- Hong Ma (MSE) half – 4 years)
- Ranjana Mehta (EE) (25% – 2 years)
- W. S. Choe (full, 2 years)
- X. Kong (Full, 2 years)
- M. S. R. Sastry (full 1 year)
- Daniel Allred (ChemE and MSE) (0.20 – 3 mos.)

16. Undergraduates (12)

Lisa Winterroth, REU
- Chris So, REU
- Bradley Newsome, REU
- Brandon Wilson, UG Research student
- Dmitriy Khatayevich, REU
- Andrea Joseph, UG Research Student
- Kevin Wang, UG Research Student. MSE 499 Project
- Jeremy Dewitt, UG Research Student, MSE 499 Project
- Peter Arrigoni, UG Research Student, REU
- Alvaro Presenda, UG Research Student, REU
- Marcus Kelly, Shorecrest High School Student – project for the Biotech Expo, 2005.. Fall/Winter 2005/2006 and Summer 2006; The student received an *Honorable Mention* award (continuing)
- Ankita Khurla, Sacred Heart High School, Issaquah, Westinghouse Science competitor.

17. Other Research Staff (2)

- Corrine Thai (F), Research Technician (Chem. Eng.)
- Eliora Gachalet (F), Research Technician (Microbiology)

18. Ph.D Awarded (5)

- Haixia Dai, Chem. Eng., Grad Student
- Daniel Allred, Chem. Eng., Grad Student
- John Kulp, III (NYU), Grad Student
- Noriyaki Suzuki, MSE, Grad Student
- Melvin Zin, MSE, Grad Student

19. Masters Awarded (4):

- Mustafa Gungormus, MSE & Mol.Biology
- Deniz Sahin, Mol. Biol.
- Lindsay Karo, Chem. Eng.,
- Arzu Ozmus, Molecular Biology and Genetics.